

Grazing and growth rate of a cyclopoid copepod fed with a phytoplankton diet constituted by a filamentous cyanobacterium

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Abstract Many tropical freshwaters experience persistent cyanobacterial blooms throughout the year. However, to date, most of the cyanobacteria–zooplankton interaction studies focused on temperate regions using large generalist grazers, which rarely coexist with cyanobacteria. Here a short-term (3 h) laboratory experiment was conducted to examine the grazing rate of *Thermocyclops decipiens* exposed to different combinations of phytoplankton (T1 = 100% *Nitzschia*-0% *Cylindrospermopsis*, T2 = 75% *Nitzschia*-25% *Cylindrospermopsis*, T3 = 50% *Nitzschia*-50% *Cylindrospermopsis*, T4 = 25% *Nitzschia*-75% *Cylindrospermopsis* and T5 = 0% *Nitzschia*-100% *Cylindrospermopsis*). In addition, an 8-day experiment was performed to determine the effect of the diet treatments on *Thermocyclops* survivorship and growth. The total biomass of phytoplankton in treatments with higher proportion of *Nitzschia* (i.e., T1 and T2) showed

abrupt decline (i.e., down to 61% and 71%, respectively) over time when compared to other treatments. While *Thermocyclops* largely excluded *Cylindrospermopsis* when *Nitzschia* is abundant, they were capable of shortening the longer filaments of *Cylindrospermopsis* into significantly smaller fragments and ingesting them when provided as a sole food. This, however, negatively influenced their survivorship and growth. Our results generally suggest that dominantly occurring selective grazers in tropical and warmer-temperate lakes may shift the phytoplankton community composition to favor cyanobacteria by grazing on the alternative food source, at least before the cyanobacteria becomes dominant.

Keywords Cyanobacteria · Cyclopoid copepod · Freshwater · Selective grazing · Tropical Lake

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Introduction

In many inland freshwater systems, eutrophication and climate change have stimulated the proliferation of cyanobacteria (O’Neil et al., 2012). Cyanobacteria often dominate the phytoplankton community composition especially in the tropics and form blooms, which may persist for a long period (Saker, 2000; Paerl & Otten, 2013). The ecological success and blooms of cyanobacteria in eutrophic waters (O’Neil et al., 2012) are attributed to a wide range of traits such as (1) superior nutrient acquisition and storage mechanisms

(Reynolds, 1984; Reynolds et al., 2000); (2) thermal and shade tolerance (O'Neil et al., 2012; Sinha et al., 2012); and (3) resistance to zooplankton grazing (Ger et al., 2014, 2016c).

Cyanobacteria have three major attributes that can constrain zooplankton grazing, survival, and growth and reproduction. Firstly, their morphological features, such as mucous layers and large size, reduce zooplankton feeding rates. In particular, the filaments can inhibit ingestion by large daphnids through clogging the filtration apparatus (Gliwicz, 1990; Gliwicz & Lampert, 1990) but not always (Panosso & Lüring, 2010). This ultimately reduces the growth rate and fecundity of zooplankton (Bednarska et al., 2014). Secondly, several cyanobacteria can produce potent toxic metabolites, which may inhibit feeding activity and cause (sub)lethal effects when ingested by zooplankton. These metabolites could be hepatotoxins, cyanotoxins, neurotoxins, and protease inhibitors (Sadler & von Elert, 2014; Ger et al., 2016a, c). The third trait of cyanobacteria that negatively affects zooplankton grazing, survival, and growth and reproduction is associated with their nutritional deficiency (Müller-Navarra et al., 2000; Müller-Navarra, 2008).

Cyanobacteria may, thus, cause major disruptions of aquatic ecosystem and lead to trophic uncoupling through negative effects on filter feeder zooplankton, such as *Daphnia* (Müller-Navarra et al., 2000), especially in the temperate zone. Nonetheless, most of the studies investigating cyanobacteria and zooplankton interactions have focused on generalist grazers (especially *Daphnia*) that rarely coexist with cyanobacterial blooms (Soares et al., 2009; Panosso & Lüring, 2010). The focus on a few large cladoceran species, however, hinders the prediction of plankton structure and interactions in systems experiencing cyanobacterial blooms and harboring zooplankton assemblages that are dominated by better adapted species, such as copepods.

Copepods, in the presence of alternative food, are shown to selectively graze against cyanobacteria (Fulton et al., 1988; Burns & Xu, 1990; Sellner et al., 1993). Selective grazing is an important attribute of copepods to withstand the blooming biomass of cyanobacteria by avoiding the ingestion of toxic cyanobacteria via grazing on a better quality prey while eluding poor-quality food items. When they encounter cyanobacteria, copepods use detection cues to avoid ingestion (Ger et al., 2011). However,

consumption of cyanobacteria could be anticipated at lower proportions of alternative food sources (DeMott, 1989; DeMott & Moxter, 1991). Indeed, this is revealed to be dependent on the toxin production and filament length of the cyanobacterial species (Ger et al., 2016b; Rangel et al., 2016). On the other hand, while some studies showed the impairment of copepod fitness by cyanobacteria (Engstrom-Ost et al., 2015), others contend that cyanobacteria do not significantly affect copepods (Ka et al., 2012) or may contribute to their feeding and reproduction (Hogfors et al., 2014). However, it has to be understood that investigations on copepod–cyanobacteria interactions mainly employed calanoid copepods as case studies (Hong et al., 2013; Engstrom-Ost et al., 2015; Hong et al., 2015).

Unlike the lakes in the temperate region, which support blooms for relatively short periods, many tropical water bodies experience persistent cyanobacterial blooms (Bouvy et al., 2001). Moreover, contrary to the temperate aquatic systems, which are characterized by large generalist zooplankton (i.e., cladocerans), tropical freshwaters are dominated by smaller size and generally selective grazers (e.g., cyclopoids) (Bouvy et al., 2001; Iglesias et al., 2010). The interactions between cyclopoid copepods and cyanobacteria, however, remain largely unexplored, especially in cyclopoid-dominated tropical systems. This has limited our understanding of the effects of cyanobacteria on the coexisting zooplankton and the responses of zooplankton to cyanobacterial blooms in tropical freshwaters. The phytoplankton and crustacean zooplankton community in Lake Ziway, Ethiopia, for instance, are dominated by cyanobacteria (mainly *Cylindrospermopsis*) and cyclopoid copepods (mainly *Thermocyclops*), respectively (Gebrehiwot et al., 2017a, b). In this lake, the highest cyanobacterial biomass (i.e., $60 \mu\text{g l}^{-1} \pm 5 \mu\text{g l}^{-1}$, $n = 3$) was recorded when the density of cyclopoids was the lowest (i.e., $15 \text{ ind l}^{-1} \pm 3 \text{ ind l}^{-1}$, $n = 3$). On the other hand, the lowest cyanobacterial biomass (i.e., $15 \mu\text{g l}^{-1} \pm 3 \mu\text{g l}^{-1}$, $n = 3$) was noted during the period of high cyclopoids density (i.e., $1662 \text{ ind l}^{-1} \pm 150 \text{ ind l}^{-1}$, $n = 3$) (Gebrehiwot et al., 2017b). This observation tempted us to study the interactions between cyanobacteria and cyclopoid copepods.

Therefore, a short-term grazing experiment was conducted to examine the grazing rate of *Thermocyclops* exposed to different combinations of

phytoplankton (cyanobacteria and diatom). In addition, an eight-day feeding experiment was performed to determine the extent to which different combinations of *Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya & Subba Raju and diatom influence the survivorship and growth of cyclopoids. We tested the hypotheses that (1) *Thermocyclops* show selection against *C. raciborskii*; and (2) *Thermocyclops* survivorship and growth depend on the proportion of *Cylindrospermopsis* in the diet.

Materials and methods

Phytoplankton collection, isolation and culturing

Phytoplankton samples were collected from Lake Ziway, Ethiopia and transported immediately to Addis Ababa University. In the laboratory, two different groups of phytoplankton (i.e., straight filament forms of *C. raciborskii* and *Nitzschia* sp.) were identified and isolated using an inverted microscope (Krüss, A. Krüss Optronic, Germany). *C. raciborskii* was isolated using a serial dilution technique (Andersen, 2005). An aliquot of a lake water sample was diluted with modified WC (MWC) medium (Soares et al., 2009) without vitamins, in a small test tube, from which drops were transferred to multi-well plates using Pasteur micropipette and concentration of individuals was checked under an inverted microscope. After a series of similar dilutions and microscopic observations, some individuals were picked up with the micropipette and introduced into multi-well plates containing MWC medium.

For *Nitzschia*, an enrichment culture was prepared by adding MWC medium to the natural sample and allowed to stand for 2 days. *Nitzschia* was carefully picked up from the enrichment culture and placed in a sterile well plate. It was then transferred to the second sterile well plate using a clean micropipette. The procedure was repeated as described in Andersen (2005) until only the required species, free of all other protists, could be obtained.

The cultures were exposed to an artificial light source from three fluorescent lamps (36 W each), providing a total photon flux density of about 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ measured on the surface of the culture, and temperatures of 22–24°C (Ogato et al., 2014) in 12-h/12-h light/dark cycle. All these cultures,

which served as sources of inoculum, were mixed manually four to five times a day.

Zooplankton collection, isolation, and culturing

Cyclopoid copepods were collected from the same lake using 50- μm mesh size plankton net. On return to the laboratory, cyclopoids were identified to the species level as *Thermocyclops decipiens* (Kiefer, 1929) and *Mesocyclops aequatorialis* Kiefer, 1929 (Sandercock & Scudder, 1994; Fernando, 2002; Witty, 2004). Since *Thermocyclops decipiens* (hereafter *Thermocyclops*) accounted for about 80% of the relative numerical abundance of cyclopoids in the lake, it was selected for the present experiments and hence isolated using a wide bore plastic pipette under a dissecting microscope. Individuals of *Thermocyclops* were then rinsed at least three times to remove other prey items (Liu et al., 2016). *Thermocyclops* cultures were maintained under identical culture conditions as those of the phytoplankton in 1 l gently aerated jars (Nogueira et al., 2006) containing equal biomass concentration of *Nitzschia* and *Cylindrospermopsis* (i.e., 50% *Nitzschia*-50% *Cylindrospermopsis*). The total concentration of food in the jar was equivalent to 101,800 $\mu\text{m}^3 \text{ml}^{-1} \sim 0.03 \text{ mg C l}^{-1}$ (Rocha & Duncan, 1985), which was comparable to the natural *C. raciborskii* and *Nitzschia* concentration in Lake Ziway (Gebrehiwot, 2018). In the process, the copepods were transferred to a fresh medium at 2-day intervals and maintained under the culture conditions for 2 weeks.

Short-term grazing experiment

To test the hypothesis that *Thermocyclops* would clear non-filamentous algae (*Nitzschia*) more readily than filamentous *Cylindrospermopsis* and that the clearance of *Cylindrospermopsis* would be dependent on its relative biomass, a short-term (3 h) grazing experiment was performed in a dark at a temperature of 22°C. This short-term incubation is expected to avoid the undesirable effects of food limitation (Panosso & Lürling, 2010) and algal growth owing to darkness (Ka et al., 2012).

Prior to this experiment, non-egg bearing and similar sized adult *Thermocyclops* were isolated and starved for about 2 h. The zooplankton used in the experiment had a mean body length of about 828 μm ($\pm 21 \mu\text{m}$, standard deviation, hereafter indicated by \pm , $n = 340$). Their body length was defined as the

distance from the most anterior part of the head to the base of the junction of the tail spine with the caudal rami.

The *Thermocyclops* were then fed with different proportions of *Cylindrospermopsis* and *Nitzschia*. For this experiment, five treatments with different proportions of the phytoplankton taxa were prepared in 12-welled culture plates by diluting exponentially growing batch cultures in the experimental medium. The treatments were T1 = 100% *Nitzschia*-0% *Cylindrospermopsis*, T2 = 75% *Nitzschia*-25% *Cylindrospermopsis*, T3 = 50% *Nitzschia*-50% *Cylindrospermopsis*, T4 = 25% *Nitzschia*-75% *Cylindrospermopsis* and T5 = 0% *Nitzschia*-100% *Cylindrospermopsis*. Each treatment had four replicates, and the well plates were filled with 2.5 ml MWC medium containing equal food concentrations of 101,800 $\mu\text{m}^3 \text{ml}^{-1}$, which is equivalent to 0.03 mg C l^{-1} (Rocha & Duncan, 1985). The aliquot of *Cylindrospermopsis* and *Nitzschia* consisted of filaments with an initial mean length of about 157 μm ($\pm 30 \mu\text{m}$, $n = 100$) and 35 μm ($\pm 5 \mu\text{m}$, $n = 100$), respectively.

The volume of water in the well plates was determined assuming an addition of a droplet of water ($\sim 0.1 \text{ ml}$) while introducing a *Thermocyclops*. Initially, the well plates that were anticipated to receive 1, 2, 4, and 10 *Thermocyclops* (described below) were filled with 2.4, 2.3, 2.1, and 1.5 ml water, respectively. The remaining volume of water was added together with the copepods.

To determine the effect of *Thermocyclops* density on their grazing rate, the experiments were conducted using different densities of *Thermocyclops*. The densities of *Thermocyclops* were 0.4, 0.8, 1.6, and 4 individuals per mL, which correspond to 1, 2, 4, and 10 individuals per well plate, respectively. The experimental densities were chosen taking the environmental cyclopid and total zooplankton densities into account, which were indicated to be as high as 2000 ind l^{-1} and 4000 ind l^{-1} , respectively (Gebrehiwot et al., 2017b). There were also four replicates of control with no animals.

During the experiment, all food suspensions were gently bubbled by a sterile-plastic pipette once every hour to minimize settling of cells (Ger et al., 2016a). For each treatment and zooplankton density, algal counts, filament length of *Cylindrospermopsis*, and biomass of the two phytoplankton were monitored hourly. This was performed by transferring a certain volume of fully

homogenized subsamples from the well plates into the Sedgwick-Rafter cell. The phytoplankton were dispersed randomly across the counting area and not localized to one particular region. The phytoplankton were enumerated using an inverted microscope (Krüss, A. Krüss Optronic, Germany). The density of each taxon from the Sedgwick–Rafter counts was calculated according to relevant literature (Wetzel & Likens, 2000; Edward & David, 2010). The volume of each phytoplankton was calculated according to the corresponding geometric configuration after measurement of appropriate dimensions (Hillebrand et al., 1999; Sun & Liu, 2003) using a digital imager (A. Krüss Optronic GmbH, Video-Okular für PC, VOP). The algal biovolume ($\mu\text{m}^3 \text{ml}^{-1}$) was then estimated by multiplying the density of each taxon by their volume. The biovolume is indicated to be equivalent to fresh weight biomass, hereafter biomass (Wetzel & Likens, 2000).

Differences in the mean biomass and filament length of *Cylindrospermopsis* during the 3-h grazing time were evaluated using repeated measures ANOVA, while one-way ANOVA was used to compare differences of the mean filament length among treatments. The clearance rate (CR) was estimated from the decrease in biomass concentration between controls and treatments according to the following equation (Peters, 1984; Panosso & Lüring 2010):

$$\text{CR} = [\ln(V_{\text{control}}) - \ln(V_{\text{treatment}})] \times t^{-1} \times m$$

where CR is the clearance rate ($\text{ml ind}^{-1} \text{h}^{-1}$), V_{control} and $V_{\text{treatment}}$ are the biomass concentration, hereafter biomass ($\mu\text{m}^3 \text{ml}^{-1}$) in the controls and treatments, respectively, after an incubation period $t(\text{h})$ and m is the volume of medium per individual *Thermocyclops* (ml ind^{-1}).

In order to test whether food composition, zooplankton density or their interaction affects the clearance rate of *Thermocyclops* on *Cylindrospermopsis* and *Nitzschia*, a two-way ANOVA, followed by Tukey's post hoc comparison test was computed. The factor food type consisted five levels as T1–T5, while zooplankton density comprised four (1, 2, 4, and 10) levels.

To assess prey selection of *Thermocyclops*, the Chesson's selectivity index (α) was calculated (Chesson, 1983):

$$\alpha_i = \left(\frac{d_i}{e_i} \right) / \left(\frac{d_i}{e_i} + \frac{d_j}{e_j} \right)$$

where i and j are prey categories, d is the proportion of prey in the diet and e is the proportion of prey in the environment.

Zooplankton growth experiment

In order to study the effect of different combinations of *Nitzschia* and *Cylindrospermopsis* on *Thermocyclops* survivorship and growth, the non-egg bearing copepodite (C5) *Thermocyclops* were cultured with different proportions of phytoplankton identical to the grazing experiment (i.e., T1–T5) for eight consecutive days. Another treatment without the food organisms in MWC medium was included as a control (T6). All treatments had the same initial phytoplankton food biomass concentration ($101,800 \mu\text{m}^3 \text{ml}^{-1}$). All treatments and the control had four replicates each.

This experiment was also conducted in 12-well plates containing 2.5 mL MWC medium and different densities of *Thermocyclops* (described above). Each treatment received similar sized *Thermocyclops* with mean body length of about $728 \mu\text{m}$ ($\pm 20 \mu\text{m}$, $n = 340$). The experiment was conducted under identical conditions as those of the culturing. The animals were transferred daily to clean well plates filled with freshly prepared food suspensions. Survival/mortality rate and growth of cyclopoids were monitored daily using an inverted microscope connected to a digital imager (A. Krüss Optronic GmbH, Video-Okular für PC, VOP). The variations in the growth of *Thermocyclops* were analyzed by means of two-way ANOVA for all treatments over the first 4 days and over the entire experimental period for T1–T4. Significant differences between mean growth were distinguished by Tukey's post hoc test ($P < 0.05$). All the statistical analyses were run using STATISTICA 13.0 (StatSoft company, Hamburg, Germany) (StatSoft, 2015). Differences at $P < 0.05$ level were considered as significant.

Results

Grazing experiment

Grazing and phytoplankton biomass

Two-way ANOVA run per *Thermocyclops* densities indicated significant effects of time ($P < 0.0001$),

food type ($P < 0.0001$), and time and food type interaction ($P < 0.0001$) on the biomass of total phytoplankton (*Nitzschia* + *Cylindrospermopsis*). The results of the grazing experiment indicated a significant decline in total phytoplankton biomass over the course of the experimental period in all treatments (repeated measures ANOVA, $P < 0.0001$; Fig. 1). The total biomass of phytoplankton in treatments with a higher proportion of *Nitzschia* (i.e., T1 and T2) showed an abrupt decline over time when compared to other treatments (T3–T5).

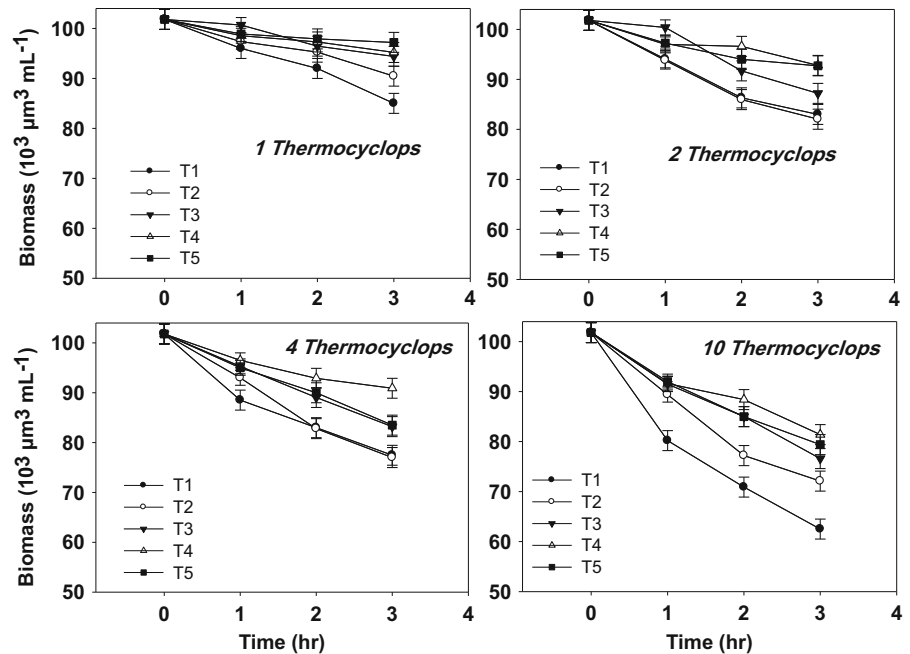
Zooplankton density also significantly influenced the biomass of phytoplankton ($P < 0.0001$). Higher densities of zooplankton per well plate expectedly led to a higher decline in phytoplankton biomass. For example, the biomass was reduced by about 40% and 25% in T1 and T2, respectively, when fed to 10 individuals of *Thermocyclops*. These figures were as low as 15% and 10% for T1 and T2, respectively, when fed to 1 individual of *Thermocyclops* (Fig. 1).

When we look at the biomass of *Cylindrospermopsis* separately, this trend is not clearly noticeable. The biomass showed a slight decline over time especially when *Thermocyclops* density was lower. Significant biomass decline of *Cylindrospermopsis* was observed only in treatments without *Nitzschia* (T5), while treatments with 25% or more of diatoms did not show a significant biomass decline (Table 1).

Clearance rate (CR) and selective grazing

Two-way ANOVA indicated a significant effect of food composition and *Thermocyclops* density on total clearance rate (TCR) of *Thermocyclops* ($P < 0.001$). The TCR on diets with a higher share of *Cylindrospermopsis* was significantly lower ($P < 0.001$) than that on mixtures with a lower share of *Cylindrospermopsis*. For all zooplankton densities, there were two homogenous groups of food types that were not statistically different from each other in terms of clearance rate. The first group comprised treatments with higher or equal proportion of *Nitzschia* (i.e., T1 = 100% *Nitzschia*-0% *Cylindrospermopsis*, T2 = 75% *Nitzschia*-25% *Cylindrospermopsis* and T3 = 50% *Nitzschia*-50% *Cylindrospermopsis*), which resulted in higher TCR. The second group consisted of the other two treatments (i.e., T4 and T5), which resulted in lower TCR. Despite being very low, the CR of *Thermocyclops* on *Cylindrospermopsis* (CR

Fig. 1 Total phytoplankton biomass (mean \pm SD) of different treatments (T1–T5) fed to 1, 2, 4, and 10 individuals of *Thermocyclops* during the 3-h experimental period; T1 = 100% *Nitzschia*-0% *Cylindrospermopsis*, T2 = 75% *Nitzschia*-25% *Cylindrospermopsis*, T3 = 50% *Nitzschia*-50% *Cylindrospermopsis*, T4 = 25% 34 *Nitzschia*-75% *Cylindrospermopsis* and T5 = 0% *Nitzschia*-100% *Cylindrospermopsis*



cyl) was detectable in treatments with higher proportions of *Cylindrospermopsis* (T4 and T5) (Fig. 2).

Comparison of the total clearance rate (TCR) at different densities of *Thermocyclops* showed that fewer individuals of zooplankton (1 or 2 *Thermocyclops*) cleared phytoplankton more readily (2–4 fold) than when the zooplankton was at higher densities. For instance, the total clearance rate of 1 *Thermocyclops* and 10 *Thermocyclops* were about 0.15 and 0.04 $\text{ml ind}^{-1} \text{h}^{-1}$, respectively, in T1. Differences in clearance rate among treatments with 1, 2, 4 or 10 individuals of *Thermocyclops* increased significantly ($P < 0.001$) with increasing proportion of *Nitzschia* in the treatments. When diets were composed of 100% *Nitzschia* (T1), differences in clearance rate among treatments were the highest (1 *Thermocyclops* = 0.15 $\text{ml ind}^{-1} \text{h}^{-1}$, 10 *Thermocyclops* = 0.04 $\text{ml ind}^{-1} \text{h}^{-1}$; hence difference = 0.11 $\text{ml ind}^{-1} \text{h}^{-1}$). However, this difference declined in treatment 5 (T5) (i.e., 1 *Thermocyclops* = 0.04 $\text{ml ind}^{-1} \text{h}^{-1}$, 10 *Thermocyclops* = 0.02 $\text{ml ind}^{-1} \text{h}^{-1}$; difference = 0.02 $\text{ml ind}^{-1} \text{h}^{-1}$) (Fig. 2).

Selection coefficient

Thermocyclops showed a clear selection preference for *Nitzschia* in all treatments with mixed diets (Table 2). The selection coefficients for *Cylindrospermopsis* in

all food items combinations were less than 0.5, which revealed feeding preference against *Cylindrospermopsis raciborskii*. But the selection coefficient for *Cylindrospermopsis* was slightly higher when the proportion of this cyanobacterium exceeded 75% of the total phytoplankton biomass (T4) (Table 2).

Filament length of *Cylindrospermopsis*

The results of experiments designed to test whether *Thermocyclops* could shorten the filament length of *Cylindrospermopsis* indicated that when it is the sole food source (T5), *Thermocyclops* could cut the longer filaments into significantly smaller pieces (repeated measures ANOVA, $P < 0.0001$). The initial experimental *Cylindrospermopsis*, which had a mean length of 157 μm ($\pm 30 \mu\text{m}$, $n = 100$), were shortened down to 120 μm ($\pm 30 \mu\text{m}$, $n = 100$) (Table 3) equivalent to 23% size reduction. This was especially noticeable at high number of grazers (*Thermocyclops*, e.g., 10). Taking T5 as an example, two-way ANOVA showed that zooplankton density ($P < 0.0001$), duration of exposure/experiment ($P < 0.0001$), and zooplankton density and time interaction ($P < 0.0001$) significantly influenced the filament length of *Cylindrospermopsis*.

Conversely, when the diet comprised a certain proportion of diatoms, the shortening of the filament of *Cylindrospermopsis* by *Thermocyclops* was not

Table 1 Mean biomass of *Nitzschia* and *Cylindropermopsis* (Cyl) in different treatments (T1–T5) in relation to the duration of exposure to different densities of *Thermocyclops* (RA-repeated measures ANOVA; ****P* < 0.0001, NS nonsignificant)

Biomass (10 ⁵ μm ³ ml ⁻¹)	1 <i>Thermocyclops</i>					2 <i>Thermocyclops</i>					4 <i>Thermocyclops</i>					10 <i>Thermocyclops</i>					
	0 h	1 h	2 h	3 h	RA	0 h	1 h	2 h	3 h	RA	0 h	1 h	2 h	3 h	RA	0 h	1 h	2 h	3 h	RA	
	<i>Nitzschia</i>																				
T1	101.8	96.1	92.0	85.1	***	101.8	94.1	86.3	82.9	***	101.8	88.5	82.9	77.5	***	101.8	80.2	70.9	62.5	***	
T2	76.4	71.9	69.8	65.0	***	76.35	68.6	60.9	57.5	***	76.35	68.1	58.1	53.0	***	76.35	65.1	54.0	50.1	***	
T3	50.9	49.8	45.6	43.5	***	50.9	49.9	42.0	39.0	***	50.9	45.2	40.1	36.2	***	50.9	42.3	37.5	33.3	***	
T4	25.5	23.3	23.1	22.9	***	25.5	22.1	22.5	21.5	***	25.5	21.7	19.3	18.5	***	25.5	20.1	18.5	17.2	***	
T5	0	0	0	0		0	0	0	0		0	0	0	0		0	0	0	0		
<i>Cyl</i>																					
T1	0	0	0	0		0	0	0	0		0	0	0	0		0	0	0	0		
T2	25.5	25.5	25.5	25.5	NS	25.5	25.5	25.5	25.5	NS	25.5	25.0	24.8	24.0	***	25.5	24.4	23.2	22.1	***	
T3	50.9	50.9	50.9	50.9	NS	50.9	50.9	50.9	50.9	NS	50.9	50.1	49.1	47.0	***	50.9	49.2	47.5	43.3	***	
T4	76.4	75.2	74.3	72.3	***	76.4	75.2	74.3	72.3	***	76.4	74.8	73.6	72.4	***	76.4	71.5	69.9	64.2	***	
T5	101.8	98.9	97.9	97.2	***	101.8	98.9	97.9	97.2	***	101.8	95.1	90.0	83.5	***	101.8	92.1	85.1	79.4	***	

Fig. 2 Clearance rate (mean \pm SD) of *Thermocyclops* for total phytoplankton (TCR), for *Cylindrospermopsis* only (CR Cyl) and for *Nitzschia* only (CR diatom) in different proportions of food items (T1–T5); T1 = 100% *Nitzschia*-0% *Cylindrospermopsis*, T2 = 75% *Nitzschia*-25% *Cylindrospermopsis*, T3 = 50% *Nitzschia*-50% *Cylindrospermopsis*, T4 = 25% *Nitzschia*-75% *Cylindrospermopsis* and T5 = 0% *Nitzschia*-100% *Cylindrospermopsis*

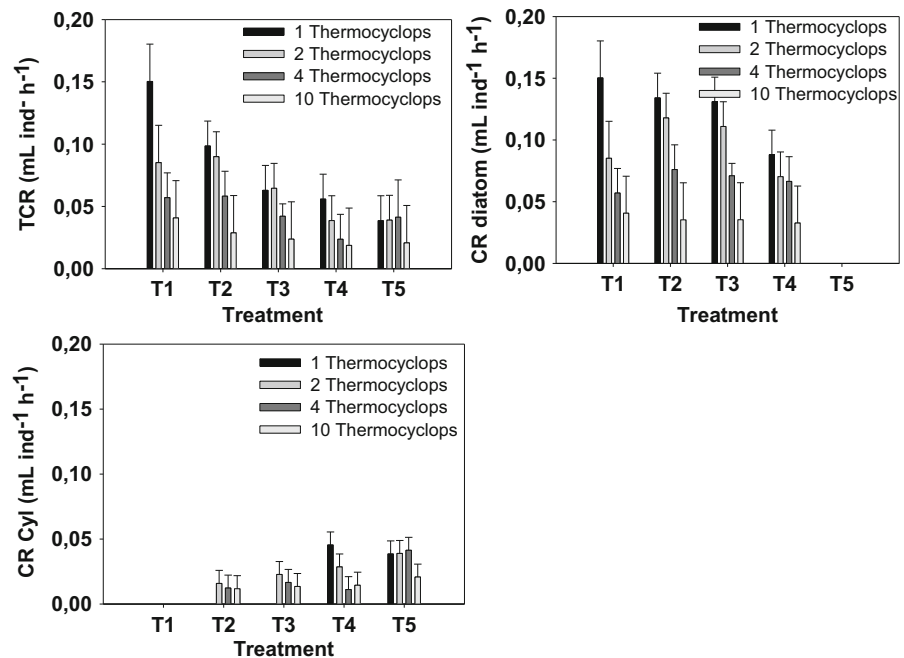


Table 2 Selection coefficient (α , mean \pm SD) at different densities of *Thermocyclops* fed with *Cylindrospermopsis* (Cyl) and *Nitzschia* in various combinations (T1–T5); NA not applicable

Treatment	1 <i>Thermocyclops</i>		2 <i>Thermocyclops</i>		4 <i>Thermocyclops</i>		10 <i>Thermocyclops</i>	
	Cyl	<i>Nitzschia</i>	Cyl	<i>Nitzschia</i>	Cyl	<i>Nitzschia</i>	Cyl	<i>Nitzschia</i>
T1	NA	1.00 (0)	NA	1.00 (0)	NA	1.00 (0)	NA	1.00 (0)
T2	0.03 (0)	0.97 (0.1)	0.04 (0)	0.96 (0.3)	0.04 (0)	0.96 (0.2)	0.04 (0)	0.96 (0.3)
T3	0.10 (0)	0.90 (0.3)	0.11 (0)	0.89 (0.3)	0.10 (0)	0.90 (0.4)	0.10 (0.1)	0.90 (0.3)
T4	0.22 (0.1)	0.78 (0.8)	0.23 (0.2)	0.77 (0.5)	0.26 (0.3)	0.74 (0.7)	0.28 (0.2)	0.72 (0.6)
T5	1.00 (0)	NA	1.00	NA	1.00 (0)	NA	1.00 (0)	NA

significant (repeated measures ANOVA, $P > 0.05$). However, in the mixed diet (T4), the filament length of *Cylindrospermopsis* was significantly shortened at the highest *Thermocyclops* density and longest duration of exposure ($P < 0.0001$, Table 3).

Thermocyclops growth experiment

Survivorship (%) of *Thermocyclops*

All the *Thermocyclops* cultured in 100% (T1) and 75% (T2) *Nitzschia* survived during the whole experimental period except those of the treatment with 10 individuals of *Thermocyclops*, in which 7.5% died since the 6th day in T2. At least 87.5% of the *Thermocyclops*

also survived during the whole experimental period when grown in T1, T2, T3, and T4. However, at the highest zooplankton density (10 *Thermocyclops* per well plate), less zooplankton survived for the whole period (Fig. 3). For instance, at the end of the experiment, the survivorship declined to 45% per well plate in T4. *Thermocyclops* also survived for slightly longer period in sole-*Cylindrospermopsis* suspension (T5) than in MWC medium with no food (T6) (Fig. 3). All the *Thermocyclops* died within four days when kept without food, while they survived for two more days (Figs. 3, 4) when fed with sole-*Cylindrospermopsis* suspension (T5).

Table 3 Filament length of *Cylindrospermopsis* (mean ± SD, μm) in different treatments (T1–T5) in relation to duration of exposure to different densities of *Thermocyclops* (RA- and OA-repeated measures and one-way ANOVA, respectively; ****P* < 0.0001, NS nonsignificant, values in bold indicate significantly lower (OA) filament length compared to other treatments, NA not applicable)

Treatment	1 <i>Thermocyclops</i>					2 <i>Thermocyclops</i>					4 <i>Thermocyclops</i>					10 <i>Thermocyclops</i>				
	0 h	1 h	2 h	3 h	RA (p)	0 h	1 h	2 h	3 h	RA (p)	0 h	1 h	2 h	3 h	RA (p)	0 h	1 h	2 h	3 h	RA (p)
T1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T2	157 (30)	157 (40)	157 (34)	157 (34)	NS	157 (30)	157 (57)	157 (50)	157 (40)	NS	157 (30)	155 (40)	155 (50)	155 (40)	NS	157 (30)	155 (60)	155 (80)	150 (80)	***
T3	157 (30)	157 (30)	157 (30)	157 (30)	NS	157 (30)	157 (69)	157 (60)	157 (50)	NS	157 (30)	155 (70)	155 (30)	155 (70)	NS	157 (30)	155 (60)	155 (60)	150 (70)	***
T4	157 (30)	157 (50)	157 (45)	157 (46)	NS	157 (30)	157 (80)	157 (60)	157 (60)	NS	157 (30)	155 (70)	155 (40)	155 (80)	NS	157 (30)	155 (70)	150 (90)	140 (80)	***
T5	157 (30)	150 (25)	148 (22)	145 (20)	***	157 (30)	150 (20)	145 (30)	143 (30)	***	157 (30)	150 (30)	140 (40)	136 (40)	***	157 (30)	150 (50)	135 (50)	120 (30)	***
OA (p)	NS	***	***	***	NS	NS	***	***	***	***	NS	***	***	***	NS	NS	***	***	***	***

Growth of Thermocyclops

The growth of *Thermocyclops* was affected by the biomass of *Cylindrospermopsis* in the diet and duration of the experiment. A two-way ANOVA of treatments with 1 *Thermocyclops* indicated significant effects of time (*P* < 0.0001), food type (*P* < 0.0001), and time and food type interaction (*P* < 0.0001) on the growth of *Thermocyclops* during the first 4 days. The same effect was also observed for treatments with two, four and ten individuals of *Thermocyclops*. The interaction effect was reflected in the progressive deviations of the body length of *Thermocyclops* among different food treatments (Fig. 4). Tukey’s post hoc test indicated that the growth of the zooplankton in T5 (0% *Nitzschia*) and T6 (no food) was significantly lower when compared to other treatments (T1–T4).

A two-way ANOVA of the entire experimental period, excluding treatments 5 (T5) and 6 (T6), also indicated significant effect of time on the growth of animals (*P* < 0.0001). Indeed, the growth of *Thermocyclops* cultured in treatments T1–T4 did not show significant variation among each other. However, they showed a relatively slower growth and shorter body length (0.82 mm ± 0.01 mm, *n* = 40) at its higher density than at its lower density (0.94 mm ± 0.01 mm, *n* = 12).

Discussion

Grazing experiment

Clearance rate and selective grazing of Thermocyclops

Copepods are indicated to coexist with blooms of cyanobacteria (Bouvy et al., 2001). However, the interactions between copepods and cyanobacteria are usually complex and controversial. Some investigators argue that copepods could negatively affect cyanobacteria (Urrutia-Cordero et al., 2015), while others produced results that showed its positive effect (Wang et al., 2010; Leitão et al., 2018).

The present study generally revealed the low clearance and strong avoidance of *Cylindrospermopsis raciborskii* by *Thermocyclops*. Specifically, in treatments with mixed diets, *Thermocyclops* cleared

Nitzschia 2–7 times faster than the filamentous cyanobacteria. The clearance rates for the total phytoplankton (TCR) and *Nitzschia* (CR diatom) declined with increased proportion of *Cylindrospermopsis* in the cultures, which implies that the cyanobacteria caused grazing inhibition.

Our present results are in agreement with the optimal diet theory, which predicts that consumers select against lower-value prey as the abundance of higher-value prey increases (DeMott, 1989). As cyanobacteria such as *C. raciborskii* lack important fatty acids and can potentially produce toxic compounds (Nogueira et al., 2004), other groups of phytoplankton are the more preferred food source than cyanobacteria for copepods. The results of stable isotope analysis by Major et al. (2017) and of the feeding experiment by Pagano (2008) also indicated the general preference of tropical zooplankton species in Ethiopia and Ivory Coast, respectively, for small seston particles ($< 20 \mu\text{m}$) (mainly green algae, flagellates and diatoms). Similarly, other researchers have noted higher selective grazing of the calanoid

copepods, *Boeckella* (Hong et al., 2013) and *Diaptomus* (DeMott & Moxter, 1991) against cyanobacteria than that of other algae. *Tropocyclops* and diaptomid copepods were also found to exhibit relatively high clearance rate when feeding on low concentrations of large algae (DeMott & Watson, 1991).

Though copepods are commonly known to feed on motile planktonic cells (Bouley & Kimmerer, 2006), they can also graze on colonial and filamentous cyanobacteria when the phytoplankton community is dominated by them (Moriarty et al., 1973; Burns & Xu, 1990; Urrutia-Cordero et al., 2015). Work & Havens (2003) also showed that copepods can graze on cyanobacteria, although other groups of phytoplankton are the preferred ones. In the present study, the greatest selection for *C. raciborskii* by *Thermocyclops* occurred when *Nitzschia* biomass was limiting/nil, which indicates the possible consumption of cyanobacteria in the absence of alternative food source. Our experiments also revealed that when *C. raciborskii* is the only food source available, *Thermocyclops* could shorten filaments of *C. raciborskii*, as

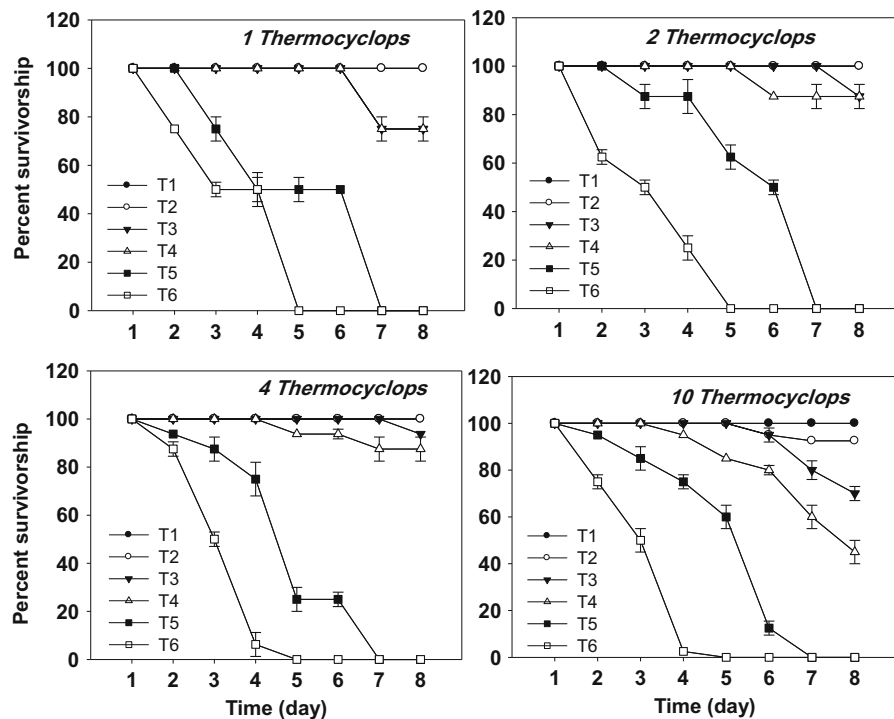


Fig. 3 Percent survivorship of *Thermocyclops* (mean \pm SD) cultured with different combinations of the phytoplankton (T1–T5) and without food (T6) during the eight-day experiment; T1 = 100% *Nitzschia*-0% *Cylindrospermopsis*, T2 = 75%

Nitzschia-25% *Cylindrospermopsis*, T3 = 50% *Nitzschia*-50% *Cylindrospermopsis*, T4 = 25% 34 *Nitzschia*-75% *Cylindrospermopsis* and T5 = 0% *Nitzschia*-100% *Cylindrospermopsis*

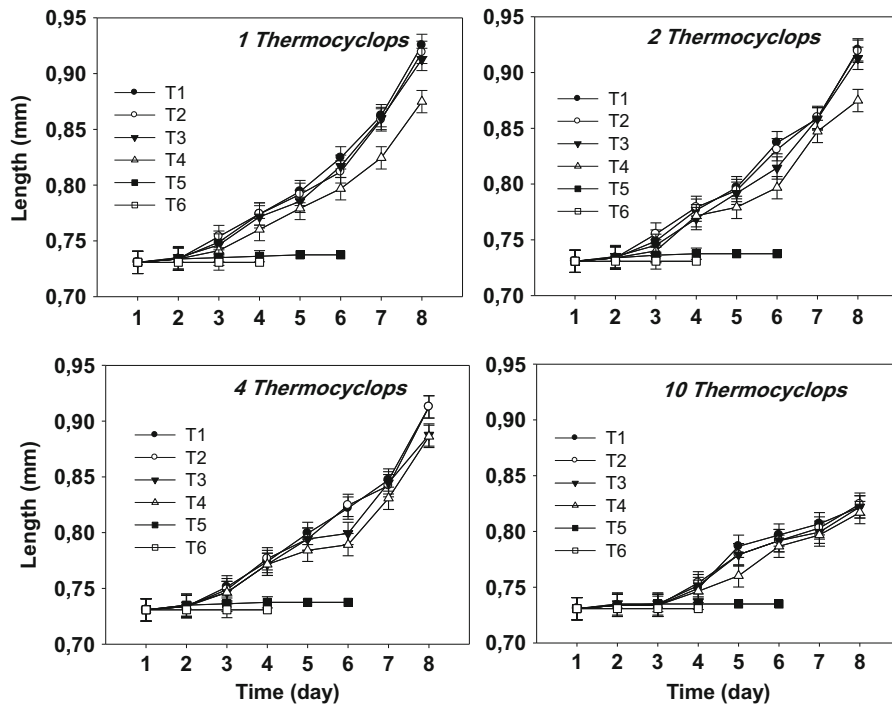


Fig. 4 Body length of *Thermocyclops* (mean \pm SD) cultured with different combinations of the phytoplankton (T1–T5) and without food (T6) during the eight-day experiment; T1 = 100% *Nitzschia*-0% *Cylindrospermopsis*, T2 = 75% *Nitzschia*-25%

Cylindrospermopsis, T3 = 50% *Nitzschia*-50% *Cylindrospermopsis*, T4 = 25% *Nitzschia*-75% *Cylindrospermopsis*, T5 = 0% *Nitzschia*-100% *Cylindrospermopsis*

illustrated by the observed reduction in the average length of its filaments. This behavior of breaking down food particles was previously noted as a feeding mechanism for calanoid copepods (Bouvy et al., 2001; Ka et al., 2012). Therefore, it can be deduced that *Thermocyclops* changes its feeding behavior depending on prey availability, being a selective consumer when the suitable food is abundant and less-selective under the limiting condition of the preferred food.

Besides the proportion of food mixtures, the density of zooplankton seems to have an important role in determining the filament length and biomass of *C. raciborskii*. Increased density of *Thermocyclops* and the consequent grazing pressure, especially when alternative prey is low, resulted in shortened filaments and reduced biomass of *Cylindrospermopsis*, which were not noted at low zooplankton density. This may be attributable to increased encounter rates at higher *Thermocyclops* density and depletion of biomass of *Nitzschia* during which *Thermocyclops* will be forced to cut and use the cyanobacterium. Ekvall et al. (2014) and Urrutia-Cordero et al. (2015) have also indicated

that high density of zooplankton is expected to influence the grazing intensity and, thus, biomass of *Cylindrospermopsis*. However, it has to be understood that the copepod density in this experimental work was higher especially while considering the natural phytoplankton–zooplankton ratio in the field (Gebrehiwot et al., 2017a, b). Hence, although our experimental results provided good insight into zooplankton density–cyanobacteria filament length and biomass relationships, it may not directly imply the natural scenario. Future researches, therefore, could focus on the effect of more environmentally relevant grazer densities on cyanobacterial filament shortening and biomass.

Generally, our results seem to suggest that selective grazing on other better quality algae may help *Thermocyclops* coexist with cyanobacteria. This is consistent with the contention that selective feeding behavior promotes grazer coexistence with cyanobacteria (Hong et al., 2013). The results also further lead us to suggest that selectively grazing *Thermocyclops* may shift the phytoplankton community composition

to favor cyanobacteria by grazing on the alternative food source, at least before the cyanobacteria becomes dominant. Hence, the inverse relationship observed between the cyclopoids density and cyanobacteria biomass in the previous studies in Lake Ziway (Gebrehiwot et al., 2017b) is not directly due to grazing. In a nutshell, our results could be extended to explain that as selective grazers are the dominant zooplankton in tropical and warmer-temperate lakes, they may facilitate cyanobacterial blooms and thus influence plankton dynamics in such systems.

Growth experiments

Survivorship of Thermocyclops

While zooplankton influence phytoplankton community, phytoplankton diets could also play a significant role in determining zooplankton community structure. Blooms of filamentous cyanobacteria, for instance, are commonly considered to impair survival and growth of grazers (Wiegand & Pflugmacher, 2005), although some investigators have reported neutral or positive effects on zooplankton (Kozlowsky-Suzuki et al., 2003).

In the present study, *Thermocyclops* was found to survive longer when fed with mixed phytoplankton. This is particularly true at lower proportions of *Cylindrospermopsis* biomass in the diet. However, significant reduction of the percent survivorship of *Thermocyclops* was observed at high *Cylindrospermopsis* proportions, especially at 100%. This might be related to the poor manageability and nutritional deficiency of the cyanobacterial species (Panosso & Lüring, 2010; Engstrom-Ost et al., 2015).

However, even though the cyanobacteria was harmful to the *Thermocyclops*, animals fed with *C. raciborskii* alone could survive for 6 days, which is slightly longer than that observed in treatments without food. This indicates that the cyanobacterial species is not acutely lethal to *Thermocyclops*, and/or it may offer some nutrition relative to the no-food treatment (Müller-Navarra, 2008; Pandey & Pandey, 2009). This finding is in concordance with the observations made by Soares et al. (2010) for *Daphnia magna*. Bouvy et al. (2001) also indicated that copepods could tolerate exposure to cyanobacterial bloom and show peak abundance in the presence of toxic cells in situ.

Growth of Thermocyclops

The growth and body length of copepods are determined partly by the quality of food (Ahlgren et al., 2005; Ger et al., 2014), which suggests that *Thermocyclops*'s growth may have also been dependent on the composition of *Cylindrospermopsis* and *Nitzschia* in this study. Accordingly, our experiments showed that as long as the food mixtures consisted of diatoms, the growth of *Thermocyclops* was not negatively affected. Even though diatoms are not as good food source as green algae owing to their frustules, they provide energy for copepod larval growth (Ianora et al., 2003). This is also possible as many copepods are able to reach high biomass in systems dominated by cyanobacteria through selective grazing of their preferred prey by size, taste and toxicity (Burns & Xu, 1990; Rangel et al., 2016).

Conversely, slower growth of *Thermocyclops* was noted in treatments consisted of sole *Cylindrospermopsis*. The results seem to suggest that harmful effects on the growth of *Thermocyclops* are expected to occur at only blooming conditions when the phytoplankton community is considerably dominated by *Cylindrospermopsis*. This is possibly because cyanobacteria, as a monospecific food, could cause oxidative stress by increasing the formation of reactive oxygen species, thereby decreasing the antioxidant capacity (Smith et al., 2008).

However, as also discussed in relation to the survivorship of copepods, filamentous cyanobacteria could have positive effect on juvenile development (Vehmaa et al., 2013). Cyanobacteria, for instance, may contain complementary nutrients and microelements (e.g., vitamin, protein, antioxidant, p and N) which are important for copepod growth (Müller-Navarra, 2008; Pandey & Pandey, 2009; Hogfors et al., 2014). Therefore, a cyanobacterium may not always be deleterious to grazers, and its ecological effects may vary depending on the presence/absence of alternative food (Jiang et al., 2010). Similar results were also recorded for a larger cladoceran, *Daphnia magna*, which was fed with *Scenedesmus* and *Cylindrospermopsis* (Soares et al., 2009).

Even though similar trends in *Thermocyclops*'s growth were noted for different densities of zooplankton, the growth was slightly slower when *Thermocyclops*'s density was increased by tenfold. This might be due to food limitation (in this experimental setup)

and associated low clearance rate. Furthermore, at the highest *Thermocyclops* density, the animals could fragment and ingest filaments of non-edible species (as suggested by a relatively higher selection coefficient), which in turn could hamper their body growth and even survival if not acute.

Conclusions

The results of the present study suggested that, within the complex interactions brought about by trophic cascades, cyclopoid copepods can ingest filamentous cyanobacteria, but select alternative food when available. However, as a sole food source, *Cylindrospermopsis* negatively influenced the survivorship and growth of *Thermocyclops*. The growth experiments conducted with either *Cylindrospermopsis* or *Nitzschia* alone, and mixture of both taxa as food organisms is believed to provide useful information on the potential ecological effect of *Cylindrospermopsis*. Given the predicted climate warming and the expected persistence of cyanobacterial blooms in the temperate region (O’Neil et al., 2012), the results of the present study on a tropical system are also believed to provide good insights into phytoplankton–zooplankton interactions in temperate zones. However, performing in situ experiments investigating cyanobacteria–cyclopoid interactions using the natural phytoplankton samples is recommended.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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